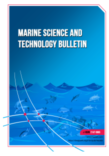
Mar. Sci. Tech. Bull. (2023) 12(4): 423-427
e–ISSN: 2147–9666
info@masteb.com

dergipark.org.tr/en/pub/masteb
www.masteb.com
DOI: 10.33714/masteb.1327138



Marine Science and Technology Bulletin

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RESEARCH ARTICLE

Presence and phylogenetic confirmation of the non-indigenous *Penaeus pulchricaudatus* (Decapoda: Penaeidea) Stebbing, 1914 in the Mediterranean Sea

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ARTICLE INFO

Article History:
Received: 13.07.2023
Received in revised form: 06.09.2023
Accepted: 07.09.2023
Available online: 28.09.2023

Keywords:
Kuruma shrimp
Penaeus japonicus
Penaeus pulchricaudatus
Phylogeny

ABSTRACT

One of the worst invasive species in terms of outspread and impact, kuruma shrimp *Penaeus japonicus* has spread subsequently in the Mediterranean basin after being misclassified for the first time as *P. canaliculatus* in Egypt. At the beginning of the 21st century, however, two morphologically or biometrically similar chromatic patterns of *P. japonicus* have been proven and clarified as two species. They do not differ morphologically, but genetically separate into form I (*P. japonicus*) and form II (*P. pulchricaudatus*). In the present study, we detect the presence and phylogenetic confirmation with mitochondrial cytochrome c oxidase subunit I (COI) gene sequences of the non-indigenous *P.pulchricaudatus* Stebbing, 1914 in the Mediterranean Sea, formerly accepted *P. japonicus*. This is the first finding in the Turkish Mediterranean waters and the first confirmation in the Mediterranean Sea.

Please cite this paper as follows:

Deval, M. C., Yılmaz, H. E., & Çağatay, İ. T. (2023). Presence and phylogenetic confirmation of the non-indigenous *Penaeus pulchricaudatus* (Decapoda: Penaeidea) Stebbing, 1914 in the Mediterranean Sea. *Marine Science and Technology Bulletin*, *12*(4), 423-427. https://doi.org/10.33714/masteb.1327138

Introduction

Penaeus japonicus (Kuruma shrimp) Spence Bate, 1888 (Decapoda, Penaeidae) recognised as the worst invasive species in terms of expansion and impacts (Pancucci-Papadopoulou et al., 2005), and distributes widely throughout the Indo-West Pacific (Pérez Farfante & Kensley, 1997; Chan, 1998). The area covers South China, Japan, Indonesian Archipelago, Australia, Red Sea, eastern and southern Africa (Dall et al., 1990). The species also crossed the Mediterranean through the Suez Canal

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(Balss, 1927; Galil et al., 2002) and reached the English Channel (Galil et al., 2002).

In the Mediterranean, after being misclassified for the first time in Egypt as *P. canaliculatus* Olivier, 1811 (Balss, 1927), the species has spread subsequently along the Levantine coasts and has been successively recorded as *P. japonicus* in southern Turkey, Syria, Israel, Greece, Rhodes, Cyprus and Lebanon (Galil et al., 2002).Other records from France, Italy (the Adriatic coast), Greece (Amvrakikos & Vistonikos Gulfs), the Sea of Marmara, Black Sea and Spain (Mar Menor), are most likely owing to escapes from aquaculture facilities (Kampouris et al., 2018). Surprisingly, almost 90 years after the first misidentification by Balss (1927), a study by Tsoi et al. (2014), using molecular and phylogenetic analyses, showed that the Kuruma shrimp has been confused for a second time (Tsoi et al., 2005) and the invasive Mediterranean population is *Penaeus pulchricaudatus* Stebbing, 1914, previously considered as a lower synonym of P. japonicus. These two forms are genetically distinct according to sequence data and restriction profiles of mitochondrial genes (Tsoi et al., 2014), although no differences were observed between the two species in terms of morphological characters or biometric parameters (Tsoi et al., 2005).

In 1979, kuruma shrimp has originated from southern Japan to Italy (Lesina Lagoon) (Lumare et al., 2000), after that, it has become important commercially cultured shrimps in the Mediterranean countries such as Türkiye, Cyprus, Greece, Albania, Egypt, Israel (Kapiris, 1997) and Italy (Quigley et al., 2013). However, it is still unknown which *Penaeus* species dominates in shrimp farms (Tsoi et al., 2007). Despite the finding that "both species do not differ in other morphological characters or biometric parameters (Tsoi et al., 2005)", there are two records of *P. pulchricaudatus* in the eastern Mediterranean based solely on images (Kampouris et al., 2018) and in situ observations during a night dive (Kampouris et al., 2019). The existence of those cryptic species that are closely related requires the genetically identification of both wild and aquaculture stocks in the Mediterranean (Tsoi et al., 2005, 2007, 2014).

The aim of this study is to distinguish two similar species of Penaeus found in the eastern Mediterranean using the molecular analysis of the cytochrome c oxidase subunit I (COI) gene region and to show that the species is *P. pulchricaudatus*. Therefore, the correct description of this species would improve aquaculture development programmes and the knowledge-based fisheries management of this economically important resource in the Turkish Mediterranean coasts (Eastern Mediterranean).

Material and Method

Shrimp individuals were collected on board R/V "*Akdeniz Su*" using a standard otter-trawl net on the commercial trawling ground in the Antalya Bay, eastern Mediterranean (Figure 1). After hauling up, the specimens were stored in ethanol for DNA isolation.

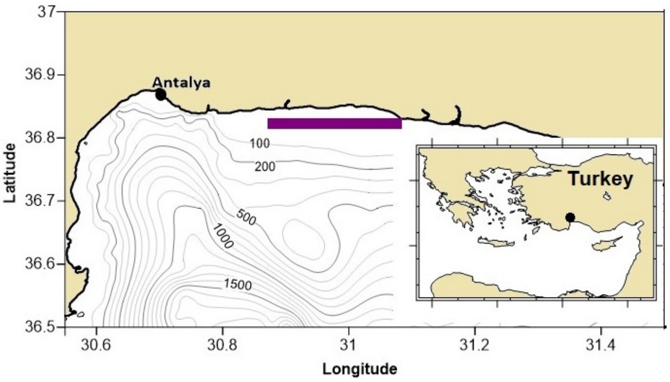

Figure 1. The study area and sampling stations in the Bay of Antalya, Türkiye (Deval, 2020)

DNA was purified from the soft muscle tissues (100 mg) using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, USA) according to the manufacturer's instruction manual. Purity and quality of DNAs were checked by running the isolated DNAs on a 1% agarose gel. DNAs were stored at +4°C.

COI gene region was used as a DNA barcode for molecular identification and phylogenetic tree construction of Penaeidae family. COIf and TL2N were used as primers for PCR amplification of this gene from Palumbi & Benzie (1991) and Quan et al. (2001). Primers were synthesized by Macrogen (Netherlands). Polymerase chain reaction (PCR) amplification of the targeted genes was performed with modifications according to Quan et al. (2001) on a Biorad thermal cycle (Biorad, USA). Amplicons of gene were screened on 1.2% agarose (Applichem, USA) and they were sequenced using same COI primers. The DNA sequencing was performed by Macrogen (Netherlands) and Medsantek (Türkiye).

CodonCode Aligner 6.0.2 software (CodonCode Corporation) was used for assembling sequences. Then consensus sequences were determined by aligning the forward and reverse sequences and matched with nucleotide database in the GenBank using BLASTn (Altschul et al., 1997). COI sequence information of species of Penaeidae family found in Mediterranean Sea from GenBank database used for phylogenetic analyses. CLUSTAL W in MEGA-X was used aligning COI sequences (Kumar et al., 2018). Pairwise distance matrices of COI gene were generated using the K2P (Kimura-2-Parameter) algorithm in MEGA-X software. The best nucleotide substitution model was defined for COI data set (GTR+G) in jModelTest v2.0 software for phylogenetic analysis (Darriba et al., 2012) under Bayesian information criteria (BIC). Bayesian inferences of gene phylogenies were carried out using Mr. Bayes (Ronquist et al., 2012). The Markov Chain Monte Carlo (MCMC) run was set to 4 chains and the analysis was carried out until the standard deviation of split frequencies fell under 0.01 (sampling every 1000 from 10,000,000 generations). The first 25% of trees were removed as burn-in and the consensus trees were visualized and coloured using Figtree v. 1.4.2 software (Rambaut, 2014).

Results

In the study, the COI gene were amplified as an 817 bp and used in the construction of the phylogenetic analyses. The COI sequences of the species in the Penaeidea family found in the Mediterranean Sea and reported in previous studies were taken from the GenBank database and included in the phylogenetic analyses. The COI dataset obtained as a result of the alignment consists of 876 characters belonging to 47 taxa. Although polytomy is seen in the phylogenetic tree constructed using the BI algorithm, the taxa are separated from each other. Our molecular analysis results and the high bootstrap values (62-100%) in phylogenetic trees (Figure 2), confirm that present sample were separated from *Metapenaeus affinis* H. Milne Edwards, 1837 and *Fenneropenaeus merguiensis* De Man, 1888 (100%) and clustered in *P. pulchricaudatus* (82%).

Discussion

Shrimp farming in Türkiye, especially along the southern Mediterranean coast, started in the early 1990s. *P. japonicus* (Japanese kuruma shrimp) was first farmed in 1995 in a semi-intensive system in Antalya (Eastern Mediterranean) (Türkmen, 2007). Two *P. japonicus* varieties I and II from the South China Sea was presented the first time by Tsoi et al. (2005) as they were morphologically very similar to each other but genetically different. According to this study, there are no definitive differences in morphometric characteristic between varieties I and II based on assessment of thirteen morphological

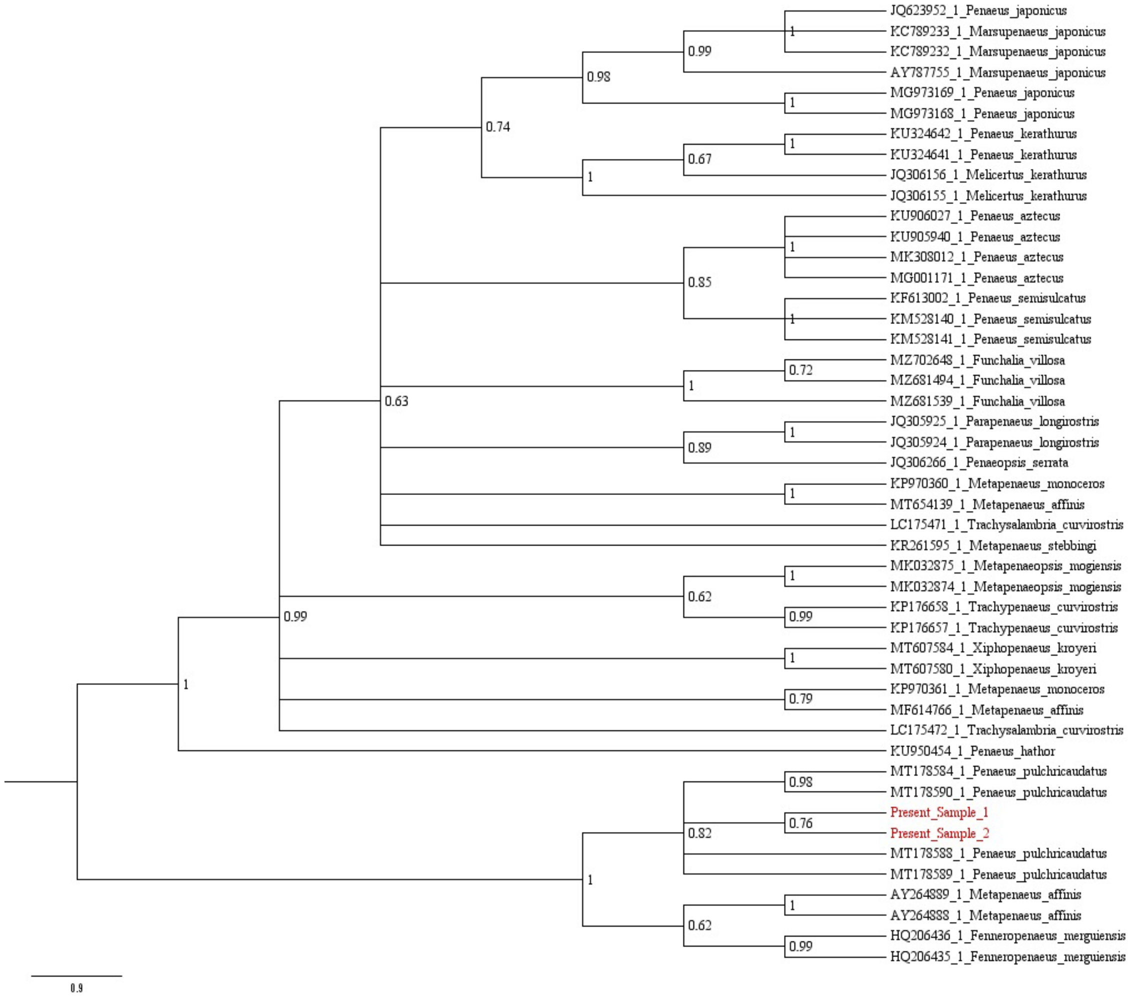

Figure 2. Molecular phylogenetic analysis using Bayesian inference for COI gene sequences

characters. Additionally, molecular analysis of the genes shows in Tsoi et al. (2005) that these two varieties separated different clades, with sequence divergences in 16S rRNA (1%), in cytochrome oxidase I (6-7%) and in the control region (16-19%).

In another study by Tsoi et al. (2007) by using the geographical distribution of these two varieties clarified ten localities based on the mitochondrial sequence analysis of the *P. japonicus*. According to his study result, variety I (*P. japonicus*) obtains populations from Taiwan, China and Japan while variety II (*P. pulchricaudatus*) consists of populations from Philippines Vietnam, Singapore, Australia, Israel (Tsoi et al., 2007).

After Israeli coasts, the result of our study confirmed that the species hitherto known as *P. japonicus* (variety I) is actually another species, *P. pulchricaudatus* (variety II) and presence also in the Antalya Bay, in the Mediterranean. *P. pulchricaudatus* is also seen that other species differ from each other in the phylogenetic tree constructed by Bayesian Inference method. Taxa within the scope of the study were separated from other species and clustered into *P. pulchricaudatus* (82%).

Since *P. japonicus* stock was presented to Italy in 1979 (Lumare, 1998), the kuruma shrimp has been continuously cultured in pond into the number of European countries such as Italy, France, Spain and Portugal (Quigley et al., 2013). However, most shrimp hatcheries still today depend upon the spawners (mature specimens) harvested from nearby wild stocks (Tsoi et al., 2014), therefore, it is unclear whether *P. pulchricaudatus* or/and *P. japonicus* is present in Mediterranean farms.

Conclusion

A wide study on the cultured shrimp in farms is essential to solve this emerging misclarification problem. Nevertheless, as in Türkiye in many other countries, it will be necessary to use *P. pulchricaudatus* instead of *P. japonicus* in fisheries statistics and many other official records.

Acknowledgements

We would like to acknowledge H. Rahmi Emral and Erkan Biçer, and the captain and crew of the R/V "*Akdeniz Su*" for their professional and diligent work at sea.

Compliance With Ethical Standards

Authors' Contributions

MCD: Sample collecting, wrote the first draft of manuscript.
HEY: Genetic analyses, Lab works and bioinformatics analyses.
İTÇ: Wrote paper, genetic analyses.
All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

Data Availability Statements

The authors confirm that the data supporting the findings of this study are available within the article.

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